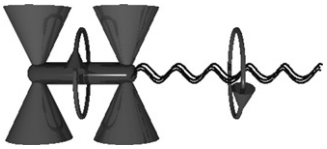
phosphorylation, and found no significant differences between NEB KO and wt muscle. Our mechanical studies revealed that NEB KO fibers had increased tension cost (5.9 vs. 4.4 pmol mN^{-1} mm^{-1} s^{-1}) and reductions in k_{tr} (4.7 vs. 7.3 s^{-1}), calcium sensitivity (pCa_{50} 5.74 vs. 5.90), and cooperativity of activation (n_H 3.64 vs. 4.38). Our findings indicate that in skeletal muscle (1) nebulin increases thin-filament activation, and (2) that through altering crossbridge cycling kinetics, nebulin increases force and efficiency of contraction. In addition to nebulin deficient murine muscle, we also studied nebulin-deficient muscle fibers from patients with Nemaline Myopathy (NM). We found increased tension cost, and reductions in k_{tr} and calcium sensitivity in NM fibers when compared to human control fibers, consistent with the findings from nebulin-deficient murine muscle. This novel role of nebulin in regulating muscle contraction adds a new level of understanding to skeletal muscle function, and might provide a mechanism for the muscle weakness in patients with nebulin-based Nemaline Myopathy.

Platform AF: Bacterial Motility

2146-Plat
High Resolution, Long Term Characterization of Bacterial Motility Using Optical Tweezers
Taejin L. Min, Patrick J. Mears, Lon M. Chubiz, Christopher V. Rao, Ido Golding, Yann R. Chemla.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.



We present a single-cell motility assay[1], which allows the quantification of bacterial swimming in a well-controlled environment, for durations of up to an hour and with a temporal resolution greater than the flagellar rotation rates of ~100 Hz. The assay is based on an instrument combining optical tweezers, light and fluorescence microscopy, and a microfluidic chamber. Using this device we characterized the long-term statistics of the run-tumble time series in individual *Escherichia coli* cells. We also quantified higher-order features of bacterial swimming, such as changes in velocity and reversals of swimming direction.
[1] Min, T.L., Mears, P.J., Chubiz, L.M., Rao, C.V., Golding, I. & Chemla, Y.R. (2009) High-resolution, long-term characterization of bacterial motility using optical tweezers. *Nature Methods* (in press)

2147-Plat
Impact of Microscopic Motility Schemes on the Overall Swimming Behavior of Parasites
Sravanti Uppaluri[1], Jan Nagler[1,2], Eric Stellamanns[1], Niko Heddergott[3], Markus Ensgtler[3], Thomas Pfohl[1,4].
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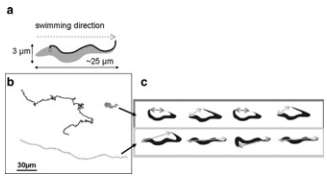

Figure 1. a) schematic of cell body b) typical swimming trajectories c) directionally persistent cells are 'stretched'

In recent work, Engstler et al. showed that the motility of the trypanosomes, causative agents of African sleeping sickness, is essential in their evasion of the host immune response. Our studies reveal that the trypanosome travels in one of three distinct motility modes: random walk, directional persistence, and an intermediate class in which they exhibit a combination of both. To further elucidate the parasite's motility we utilize high-speed videomicroscopy to uncover the microscopic origin of the macroscopic motility modes. Trypanosome swimming is facilitated by a flagellum that runs along the cell body with only a small 'free' segment at the anterior end of the cell. We use a straightforward parameter, namely the distance between the anterior and posterior ends of the cell to characterize trypanosome swimming. Remarkably this parameter is sufficient for extraction of relevant time scales for classification of the motility modes. Further, we find not only that these different motility modes correspond to distinct physical movements but also that a stiffer cell body gives rise to directional persistence.

2148-Plat
A Model for Bacterial Motility Utilizing Helical Cytoskeleton Filaments and Ion-Driven Motors
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The bacterial cytoskeleton determines cell shape and mediates cell division. Recent work indicates that the cytoskeleton mediates cell motility as well. Adventurous (A) motility in Myxococcus xanthus requires actin-like MreB filaments. During the motility, a double helix structure – possibly consisting of MreB – rotates in the cell's cytoplasm. Proteins localized along the helical structure are associated with proton transporting protein complexes homologous to the MotA-MotB stator that drives rotation of the bacterial flagellar motor. These observations suggest an entirely new model for bacterial motility in which motors driven by ion motive force move along the helical cytoskeleton to generate propulsive forces. This mechanism may be widespread in bacteria, since both MreB homologs and MotA-MotB homologs are common across a variety of bacterial species, including species that move in the absence of flagella. We have constructed a biophysical model to test the feasibility of this motility mechanism. Our model explains many intriguing observations in Myxococcus motility, including rotation of the helical cytoskeleton, periodic reversals of cells, and clustering of motility-related proteins at the cell poles and the substrate interface. Previous models assumed that periodic cell reversals are attributed to biochemical oscillators in the cell. This model, in contrast, proposes that reversals result from a mechanical oscillator intrinsic to a system with transmembrane motors traveling on a closed helical track. According to this mechanism, mechanical interactions play an important role in signal transduction.

2149-Plat
Direct Evidences of a Motility Motors in Myxococcus Xanthus
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Myxococcus xanthus, a gram-negative soil bacterium, glides over solid surfaces with two independent motility mechanisms termed social (S) and adventurous (A) motility. S-motility has been shown to be powered by the extrusion, adhesion and retraction of type IV pili. A-motility, however, is much less well understood. Two main models have been proposed to explain A-motility. The first, the ''slime gun'' model, implicates the secretion of polyelectrolyte slime from the cell pole, pushing a cell forward. More recently, we proposed a second model that involves lateral force generation at focal adhesion sites between the cell and substrate. In this model, unknown motors drive cells forward by moving along a filament inside the cell. To date, however, there has been no direct evidence for lateral force generation or the presence of motor proteins in Myxococcus.
We have developed an optical trapping bead assay, which enables us to adhere polystyrene beads to the surface of Myxococcus cells. We find that beads are moved along the cell surface with speeds comparable with those of gliding cells. Beads move in a helical pattern along the cell surface with a pitch size of a few microns. Multiple beads on the same cell can move in the different directions, indicating that beads are carried by individual motors instead of by a global movement of the cell envelope. We also show that in mutant cells lacking key A-motility regulatory genes, beads move in a less coordinated fashion.

2150-Plat
Morphogenesis and Cell Division of E. Coli Under Mechanical Confinement
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Bacteria have characteristic shapes and sizes which are conserved by an elaborate cytoskeletal machinery. Surprisingly, these well-defined shapes are strongly modified in E. coli bacteria in narrow nanofabricated channels. Growth in constrictions where bacteria are squeezed to about twice thinner than their typical diameter leads to flattened cells that laterally are much wider (up to 5 micron) than regular E. coli [1]. We will report on the cell growth, spatial structure and dynamics of cytoskeletal proteins and the nucleoid in this unusual bacterial phenotype. While the physical confinement has a profound effect on the cell shape and the pattern of cell division, it has only a limited effect on the replication rate of the cells. In most cases, broad (multinucleate) cells are still able to segregate chromosomes in roughly equal amounts to two daughter cells. This process typically starts with the formation of a chromosome-free area in the middle of the cell, which propagates asymmetrically to the perimeter of cell.